Extracellular Matrix Proteins (Fibronectin, Laminin, and Type IV Collagen) Bind and Aggregate Bacteria

G. M. VERCELLOTTI, MD, J. B. McCARTHY, PhD, P. LINDHOLM, MD, P. K. PETERSON, MD, H. S. JACOB, MD, and L. T. FURCHT, MD

From the Departments of Medicine and Laboratory Medicine and Pathology, University of Minnesota, Minneapolis

The normal microbial colonization of sites in the body's tissues by certain bacteria requires that the bacteria first bind to extracellular secreted constituents, cell-surface membranes, or cell matrixes. This study examines two interactions of a variety of bacteria with the cell matrix noncollagenous proteins fibronectin and laminin and with basement membrane (Type IV) collagen. Adherence of bacteria to matrix proteins coated on tissue culture wells was examined with the use of radiolabeled bacteria. *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus sanguis* bound well to fibronectin, laminin, and Type IV collagen, whereas a variety of gram-negative organisms did not bind. The interaction of soluble laminin, fibronectin, and Type IV collagen with bacteria was monitored by nephelometry with the use of a platelet aggregometer. *S. aureus* aggregated in response to fibronectin, laminin, or Type IV collagen. In contrast, gram-negative organisms did not aggregate with these proteins. It appears that fibronectin, laminin, and Type IV collagen can bind and aggregate certain gram-positive bacteria, and this binding is dependent on the surface characteristics of the organism. These adhesion molecules may play a role in the normal colonization of sites by microorganisms and in invasion during infections. (Am J Pathol 1985, 120:13–21)

A CRITICAL EVENT in the initiation of a variety of bacterial infections involves the adherence of bacteria to epithelial and endothelial cell surfaces. The process of adherence depends on a recognition system whereby a bacterial surface ligand binds to a specific host cell receptor as a quasi-specific binding site leading to adhesion. The bacterial surface ligands differ for gram-positive and gram-negative organisms on the basis of cell surface characteristics.[1,2] Gram-positive organisms have cell walls consisting of proteins, teichoic acid, peptidoglycans, lipoteichoic acid,[3] and polysaccharides, all of which have been implicated in mediating cell attachment. Gram-negative organisms have a lipopolysaccharide-rich outer membrane and utilize fimbriated pili[4] and a glycocalyx to mediate adhesion to various biologic surfaces.

The extracellular substrate for bacterial adhesion has recently been addressed. Cell fibronectin binds to *Staphylococcus aureus* via a site in the NH_2-terminal, 27-kd fragment of fibronectin.[5] The binding is noncovalent, dependent on culture conditions of the bacteria and pH,[6] and can be inhibited by high concentrations of glucuronic acid, heparin, and basic amino acids.[7] Fibronectin can clump a variety of *S aureus* strains but not *Staphylococcus epidermidis*.[8,9] The fibronectin receptor on *S aureus* is a protein but is not protein A.[10,11] Fibronectin binds to streptococci via bacterial lipoteichoic acid,[12] and fibronectin can mediate the attachment of streptococci to neutrophils[13] and epithelial cells *in vitro*.[14] In contrast, gram-negative organisms do not bind to epithelial cells that have fibronectin on their surface but do readily attach to the cells if they are relatively devoid of surface fibronectin.[14,15] We recently demonstrated similar results, utilizing the fibronectin-rich vascular endothelial cell as a substrate for bacterial adherence.[16] That is, certain bacteria would readily adhere to endothelial cells cultured *in vitro*.

The pathogenesis of bacterial infection involves attachment to and penetration of bacteria through basement membrane structures that underlie epithelial and endothelial cell linings. In recent years, much informa-

Supported in part by NIH Grants HL19725 and HL28935; Minnesota Medical Foundation grant MRF19-82; a grant from the American Heart Association, Minnesota Affiliate, Inc.; and grant CA2999 from the Leukemia Task Force. LTF is Stone Professor of Pathology, University of Minnesota, and a recipient of Research Career Development Award CA00651 from NCI/NIH.

Preliminary results were presented at the Midwest AFCR Meetings in Chicago in November 1983.

Accepted for publication February 11, 1985.

Address reprint requests to Gregory M. Vercellotti, MD, Box 480 Mayo Memorial Building, University of Minnesota Hospitals, 420 Delaware Street SE, Minneapolis, MN 55455.

tion has been gained concerning the nature of the constituents that make up basement membranes. These components are collagens, noncollagenous glycoproteins, and proteoglycans. The major noncollagenous adhesive glycoprotein in basement membranes is laminin.[17,18] This protein has an extremely high molecular weight, with estimates of up to 1×10^6 daltons. The protein has disulfide-bonded subunits of 200 kd and 400 kd, which are organized into an asymmetric cross-shaped structure seen by rotary shadowing.[18] Laminin has been reported to bind to basement membrane (Type IV) collagen as well as to glycosaminoglycans. Additionally, laminin promotes the adhesion and spreading of a variety of cell types[19-21]; and recently it has been shown to promote the motility by haptotaxis of tumor cells *in vitro*.[22,23] The hypothesis is, as is the case with fibronectin in interstitial tissues, that laminin appears to have a pivotal role in basement membranes by promoting the adhesion of cells to the structural framework of the basement membrane. Basement membranes contain Type IV collagen,[18] which consists of two distinct chains, α1(IV) and α2(IV) with apparent molecular weights of 185,000 and 170,000 (See review[24] and references therein). Type IV collagen is distinct, compared with the interstitial (Type I) collagen. Both collagens are helical and contain the collagenous glycine X-Y (Y is frequently hydroxylysine or hydroxyproline) sequence that defines the collagenous helix; however, Type IV collagen has this sequence interrupted twice with noncollagenous globular domains. Type IV collagen does not form striated fibrils, as does Type I collagen, but instead forms a network type of structure that is apparently important for basement membrane organization.[25] Compared with fibronectin, little work has been published concerning the interaction of laminin and Type IV collagen with bacteria. Speziale[26] reported that laminin binds to *Escherichia coli* but not *S aureus*, suggesting that laminin may be an important substrate for bacterial adherence and interacts distinctly from fibronectin with bacteria.

Since colonization and invasion are characteristic features of the pathogenesis of many bacterial infections, it is reasonable to compare the affinity of certain bacteria with cell matrix proteins. This study examines the interaction of various gram-positive and gram-negative organisms with surfaces coated with fibronectin, laminin, and Type IV collagen: the gram-positive organisms, including *S aureus, S pyogenes,* and viridans streptococci, and the gram-negative organisms, including *E coli, Enterobacter cloacae, Klebsiella pneumoniae,* and *Serratia marcescens.* Because there are differences in the ability of eukaryotic cells to respond to soluble or substrate-attached matrix constituents, the ability of bacteria to interact with soluble laminin, fibronectin, and Type IV collagen was studied in a novel manner, based on bacterial agglutination, with the use of a platelet aggregometer. This study demonstrates that gram-positive, but not gram-negative, organisms avidly bind to these matrix-protein–coated surfaces and that a representative gram-positive organism, *S aureus*, aggregates in the presence of fibronectin, laminin, and Type IV collagen.

Materials and Methods

Bacterial Strains and Culture Conditions

Laboratory strains of *S aureus* Cowan I, Wood 46 (a protein-A–deficient strain) and 52A5 (a teichoic-acid–deficient mutant of HSMR provided by J. T. Park, Tufts University, Boston, Mass) were utilized. *S sanguis* organisms were provided by W. Liljemark, University of Minnesota. *S pyogenes* organisms were kindly provided by the late L. Wannamaker, University of Minnesota. Gram-negative strains of bacteria were obtained from the blood of bacteremic patients. These included *E coli, K pneumoniae, E cloacae,* and *S marcescens.* All strains were maintained on blood agar plates at 4 C. For each experiment bacteria were freshly grown in Mueller–Hinton broth (pH 7.4) with 2 μCi/ml ^{3}H-thymidine (6.7 Ci/mM, New England Nuclear, Boston, Mass). The bacteria for aggregation experiments were not radiolabeled. After incubation at 37 C in a shaking incubator for 16 hours, the bacteria were harvested, washed three times with phosphate-buffered saline (PBS), pH 7.4, and resuspended in Hanks' balanced salt solution (HBSS), pH 7.4, with magnesium, calcium, and 0.5% human albumin (Travenol Laboratories, Inc., Glendale, Calif). Initially, bacterial counts were obtained in a Petroff–Hauser chamber and used to calibrate a Coleman Junior Spectrophotometer (Coleman Instruments, Maywood, Ill). Cell counts were estimated spectrophotometrically thereafter.

Purification of Cell Matrix Proteins

Human plasma fibronectin was isolated from a fibrinogen-rich byproduct of Factor VIII production by the use of a DEAE ion exchange column followed by gelatin affinity chromatography as previously described.[27] Elution of plasma fibronectin from the gelatin–agarose column was performed with the use of 0.02 M sodium acetate, pH 5.0, with 1 M NaBr. Laminin and Type IV (basement membrane) collagen were purified from the EHS tumor as previously described.[28-30] Briefly, laminin was extracted from the tumor with the use of neutral salt. Laminin was purified over a Sephadex S-200 column and a heparin affinity column. The EHS tu-

mor grown in lathyritic animals was used as a source for Type IV collagen.[29] The laminin was first removed from the tumor with the use of neutral salt, and Type IV collagen was subsequently extracted with the use of 0.05 M Tris-HCl, pH 7.4, containing 2.0 M guanidine-hydrochloride and 1.6 mg/ml dithiothreitol. Contaminating acidic glycoproteins were removed from the extract by chromatography over a DEAE column as described.[29] The purity and integrity of the resulting purified proteins was judged by discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis.[31] Fibronectin appeared as a closely spaced doublet on reduction with mercaptoethanol with molecular weights of 220 and 240 kd. Reduction of laminin revealed two subunits of 400 kd and 200 kd. Type IV collagen appeared as a closely spaced doublet of 185 kd and 170 kd upon reduction. Purity was then assessed with an enzyme immunoassay[27] using affinity purified antibodies which had been absorbed against potential cross-reacting antigens (eg, anti-laminin antibodies were absorbed on Type IV collagen affinity columns).

Bacterial Aggregation Assay

The bacteria to be tested were prepared in a stock suspension by dilution with HBSS. Using a Payton dual channel aggregometer (Buffalo, NY) for each aggregation trial, we added 450 μl of the bacterial suspension (2×10^9/ml) to siliconized cuvettes with a stirring bar at 600 rpm at 37 C. Purified matrix proteins were precentrifuged at 5000*g* for removal of aggregates; the proteins or bovine serum albumin (BSA) was added in various concentrations in a volume of 50 μl to the stirring bacterial suspension. The proteins added in these concentrations to cuvettes not containing bacteria had no visible signs of protein aggregation. Aggregation was recorded on a Payton recorder at a paper speed of 2.5 cm/min.

Pretreatment of Bacterial Suspension With Heparin

Heparin sulfate (Upjohn, Kalamazoo, Mich), 1000 U/ml, was diluted appropriately and added in 50-μl aliquots to the stirring suspensions of bacteria for 5 minutes prior to aggregation. The final concentration ranged from 0.5 U/ml to 50 U/ml of heparin.

Coating of Substrates With Protein

Briefly, solutions of fibronectin, laminin, Type IV collagen, and BSA were added to 2-sq cm tissue culture wells (Costar, Cambridge, Mass) for 12 hours at 37 C with the indicated concentrations of each of these proteins in carbonate buffer (pH 9.6). The plates were then washed with buffer. To ensure attachment of these proteins, in separate studies we tritiated the proteins by reductive methylation[28] with ^{3}H-formaldehyde. Free radioactivity was removed by dialysis, and labeled proteins were electrophoresed on SDS-PAGE and examined by autoradiographic analysis. Radioactive proteins were then added, in carbonate buffer, to 24-well plates and incubated overnight. Unbound protein was washed with PBS, and bound protein was solubilized 0.5 N NaOH with 1% sodium dodecyl sulfate. Bound radioactivity was quantitated in a Beckman 3801 liquid scintillation counter.

Bacterial Adherence Assay

Prior to performing the bacterial adherence assay, bacteria were suspended in HBSS with 0.5% human albumin at a concentration of 10^8 organisms/ml. Wells in plates coated with fibronectin, laminin, Type IV collagen, and BSA were washed with the same buffer immediately prior to use. A bacterial suspension (0.5 ml) was added to each well, and each individual sample was assayed in triplicate. The total amount of bacteria-associated radioactivity that was added to each was measured separately. Tissue-culture plates with the added bacteria were placed in a stationary 37 C incubator and allowed to incubate for 2 hours. After incubation the wells were washed three times, and the radioactivity of the nonadherent bacteria was measured in a liquid scintillation counter. The tissue-culture wells were then treated with 0.5 ml of 0.05% trypsin–1 mM EDTA for release of the adherent bacteria and assayed separately. There was good correlation between the radiolabel recovered (supernatant and adherent) and total radiolabel added. Microscopic examination of the wells ensured complete removal of all the bacteria. The percent bacterial adherence equaled the cpm adherent bacteria/cpm(total) $\times$ 100. Background bacterial binding to uncoated plastic was subtracted from each assay for calculation of substrate adherence.

Proteolytic Treatment of Bacteria

Bacteria were suspended in HBSS containing trypsin 1 mg/ml (Type IX, Porcine Pancreas, Sigma Chemical Company, St. Louis, Mo) for 30 minutes at 37 C. After incubation, the bacteria were washed three times with HBSS and resuspended in buffer at the indicated concentrations for use in either the adherence assay or the aggregation assay. In a number of experiments after trypsin treatment and washing, soybean trypsin inhibitor (Sigma) was added at a tenfold molar excess to the bacteria for trypsin inhibition. Bacteria were then

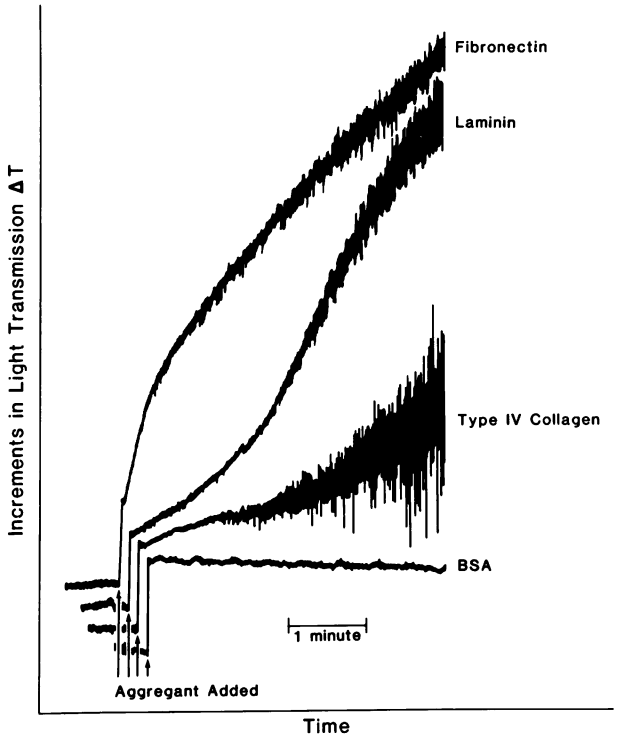

Figure 1—Aggregation of *S aureus* Cowan I by fibronectin (200 μg/ml, final), laminin (100 μg/ml), Type IV collagen (100 μg/ml), and BSA (100 μg/ml). The protein is added to the *S aureus* Cowan I (2 × 10^9/ml) stirring in a cuvette at 600 rpm at 37 C. The ordinate is in increasing increments of light transmission, and the abscissa is time in minutes.

washed two more times and utilized in the adherence assay or the aggregation assay. No direct effect of soybean trypsin inhibitor on bacterial adhesion or aggregation was noted.

Photomicrographs

While being stirred at 600 rpm in the aggregometer, samples of 2 × 10^9 bacteria/ml were taken before and after the addition of a matrix protein for microscopic examination of the aggregation of bacteria. A 20-μl sample was placed on a glass slide and allowed to air-dry. The slides were then stained with Gram stain and examined microscopically.

Results

Bacterial suspensions were first qualitatively tested for aggregation with the proteins fibronectin, laminin, Type IV collagen, and BSA. *S aureus* Cowan I aggregated markedly with the addition of the matrix proteins but not with BSA (Figure 1). Fibronectin and laminin showed the greatest aggregating effect. Type IV collagen was substantially less than either fibronectin or laminin, but was consistently greater than observed with the BSA control. Dose-response data for fibronectin shows minimal aggregation at a concentration of

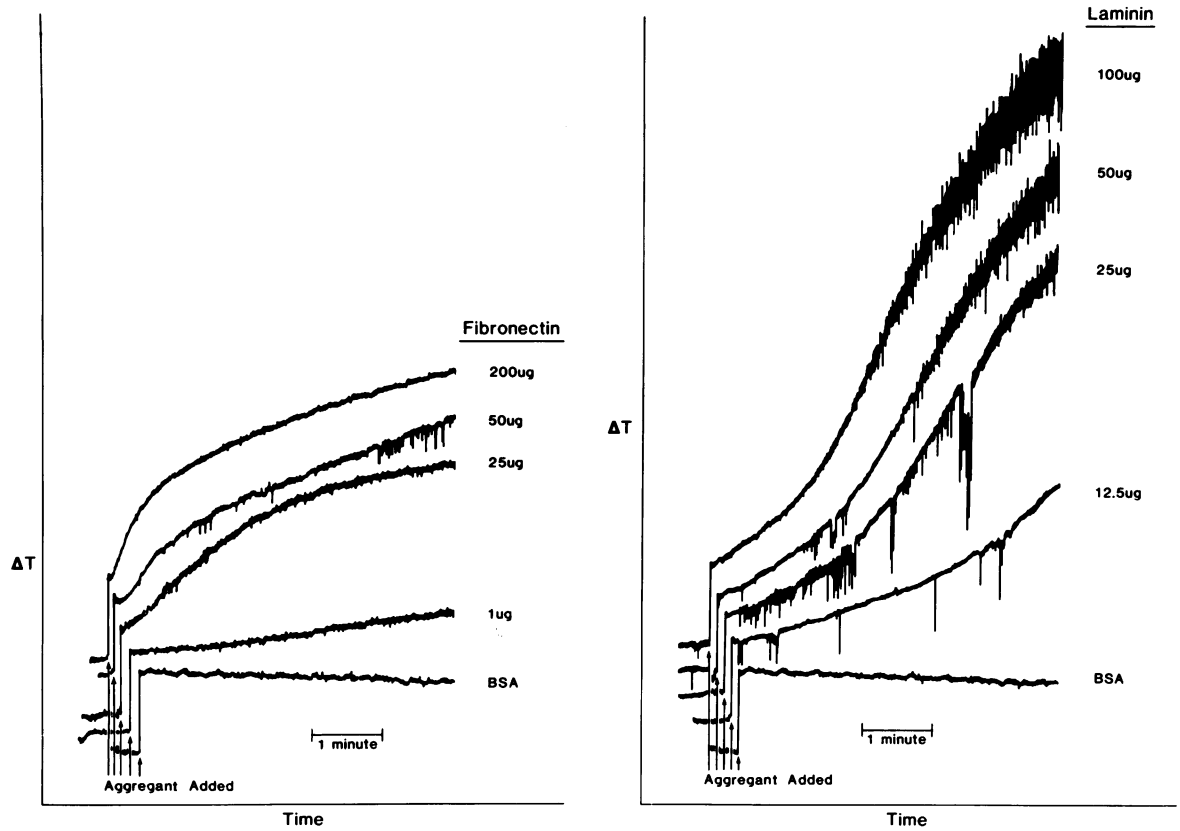

Figure 2—Aggregation of *S aureus* Cowan I by increasing final concentrations of fibronectin and laminin. As in Materials and Methods (left panel), increasing concentrations of fibronectin are added to *S aureus* Cowan I stirring in a cuvette. The right panel reveals increasing concentrations of laminin.

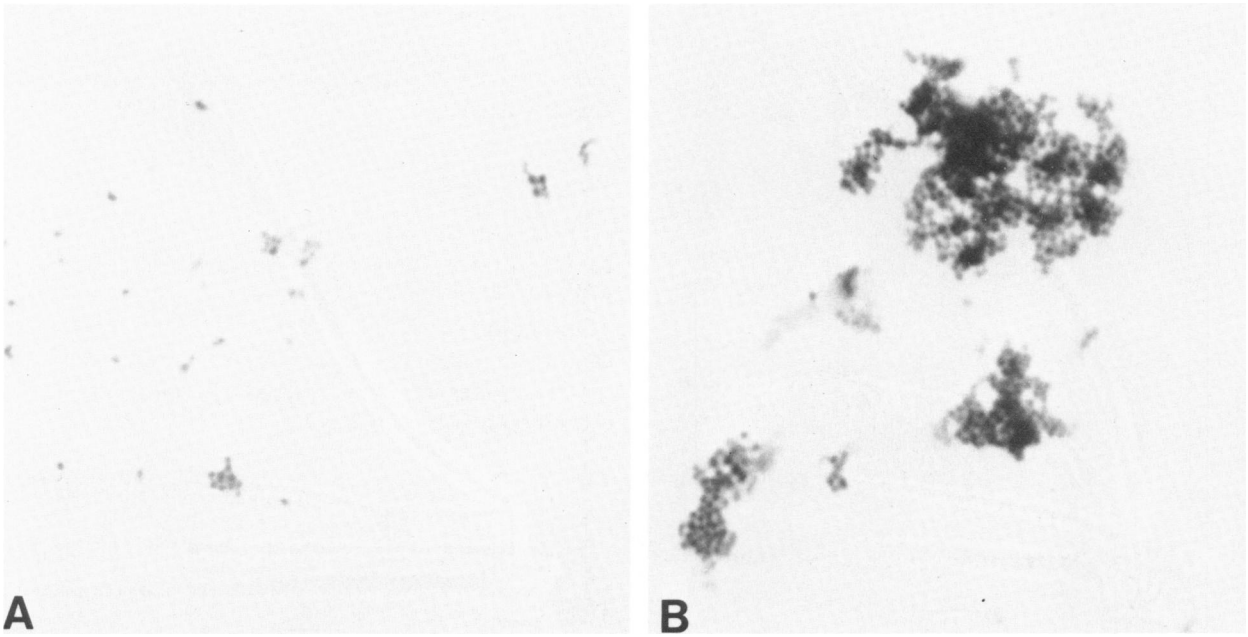

Figure 3—Photomicrographs of untreated *S aureus* (Cowan I strain) (**A**). **B** reveals clumps of *S aureus* after the addition of fibronectin (200 μg/ml, final concentration), Type IV collagen (100 μg/ml), or laminin (100 μg/ml). The bacteria being stirred in the cuvette underwent addition of the matrix proteins for 2 minutes. A sample of the bacterial suspension was then removed and placed on a glass coverslip as described in Materials and Methods, and the bacteria were stained with Gram's stain. **B** was taken at × 1000 and represents bacteria aggregated with fibronectin.

1 μg/ml (Figure 2, left panel) with aggregation increasing proportionately up to 200 μg/ml. Laminin caused nominal aggregation of *S aureus* at 12.5 μg/ml (Figure 2, right panel) with increasing aggregation observed at up to a 100-μg/ml final concentration. Type IV collagen aggregated *S aureus* at slower rate, compared with fibronectin or laminin, in concentrations from 10 μg/ml to 200 μg/ml (data not shown). Microscopic observation of the bacteria under the various conditions revealed individual cells or small clusters of 4–16 staphylococcal cells when BSA was added (Figure 3A), whereas clusters of 50 to several hundred bacteria were seen when fibronectin (50 μg/ml) (Figure 3B), laminin (50 μg/ml), or Type IV collagen (50 μg/ml) was added.

The effect of certain surface components of *S aureus* were studied by comparing the aggregation of the Cowan I strain, *S aureus* mutants deficient in either teichoic acid or protein A, or Cowan I pretreated with trypsin (Figure 4). Fibronectin did not aggregate protein-A–deficient *S aureus* Wood 46 or the trypsin-treated Cowan I strain. There was slight aggregation with the teichoic-acid–deficient strain 52A5. Similarly, laminin caused little aggregation of the teichoic-acid–deficient strain and no aggregation of the protein-A–deficient or trypsin-treated *S aureus*. In data not shown, Type IV collagen did not aggregate protein-A–deficient, trypsin-treated, or teichoic-acid–deficient *S aureus*.

Because heparin interacts with certain cell matrix proteins, its effect on bacterial aggregation was next examined. As seen in Figure 5 (right panel), 0.5–50 units of heparin added to the bacterial suspension decreased the rate but did not completely block aggregation to fibronectin. Heparin also had a similar effect on inhibiting bacterial aggregation both by laminin (Figure 5, right panel) and Type IV collagen (not shown).

A variety of other bacteria were examined for their ability to aggregate with matrix proteins: *S pyogenes*, *S sanguis*, and gram-negative organisms (including *E coli*, *K pneumoniae*, *S marcescens*, and *E cloacae*) did not aggregate with fibronectin or laminin (Figure 6, right and left panels).

The adherence of gram-positive and gram-negative organisms was next tested on surfaces coated with fibronectin, laminin, Type IV collagen, and BSA. Time-course and dose-response data on adherence of *S aureus* to matrix-protein–coated wells revealed that there was increasing adherence with increasing duration of incubation of staphylococci up to 6 hours. A 2-hour time point was chosen for subsequent experiments because consistent specific adherence was observed. A dose-response test for the amount of matrix protein coating the plate was done, and preliminary experiments indicated (data not shown) that the optimal coating concentrations for attachment were 50 μg of fibronectin, (0.7 μg/well residual protein measured as in Methods), 25 μg of laminin (4.5 μg residual protein/well), and 50 μg Type IV collagen (10.8 μg residual protein/well). *S aureus* (Cowan I strain) adherence to uncoated plastic was negligible (2.5% ± 0.2%). Adherence to BSA (2.2% ± 0.2%) was also minimal, compared with adherence to fibronectin (23% ± 2%), laminin (22% ± 2%) or Type IV collagen (21% ± 3%) (Table 1). It could

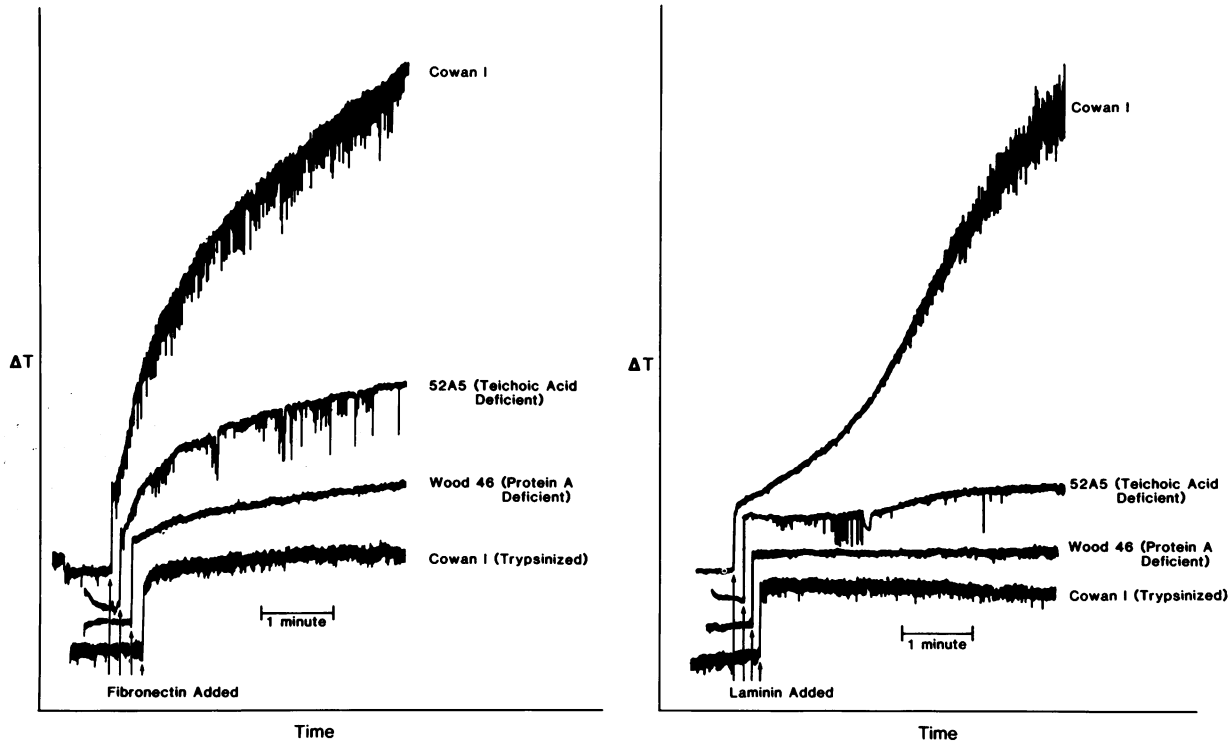

Figure 4—Aggregation of various strains of *S aureus*. In the left panel is shown aggregation of 52A5 (a teichoic-acid–deficient strain of *S aureus*), Wood 46 (a protein-A–deficient strain of *S aureus*), and Cowan I that had been exposed to trypsin (as described in Materials and Methods). In each experiment, 450 μl of a suspension of 2 × 10^9 bacteria/ml were utilized. A final concentration of 200 μg/ml of fibronectin was utilized in each experiment. Similarly (right panel), 100 μg/ml laminin was added to these same bacteria.

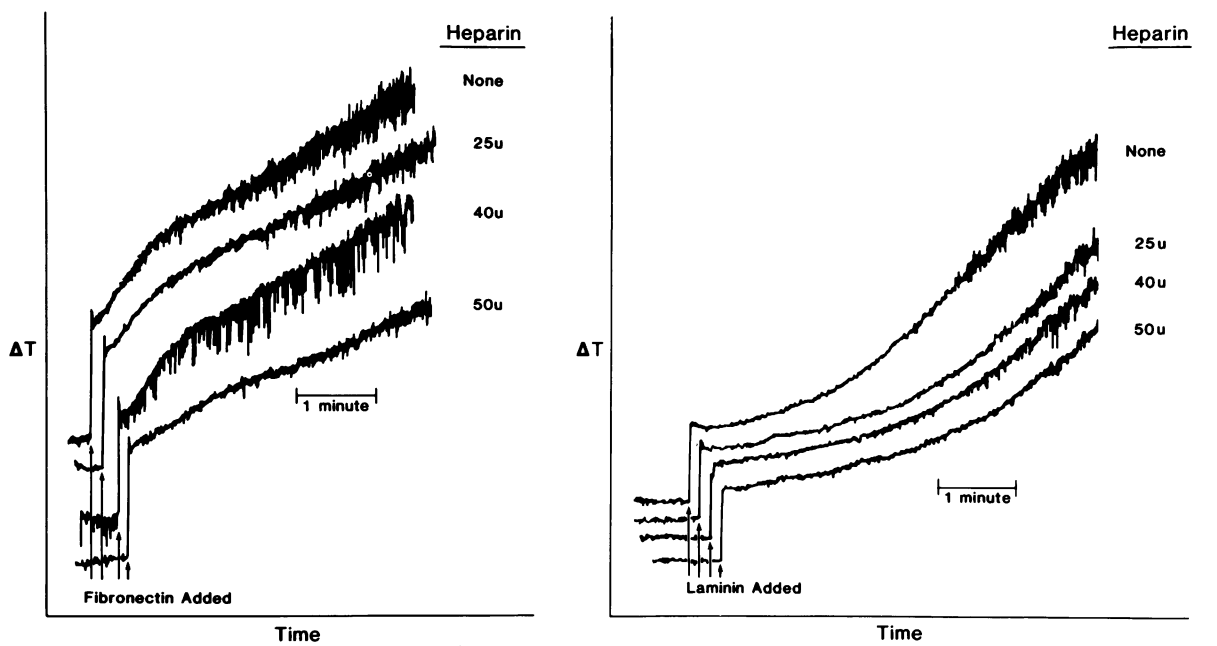

Figure 5—Effect of heparin on *S aureus* aggregation. A final concentration of 200 μg/ml fibronectin was added to *S aureus* Cowan I that had been preincubated for 2 minutes with varying amounts of heparin, as indicated in Materials and Methods (left panel). Similarly, laminin (50 μg/ml final) was added to *S aureus* (Cowan I) after incubation with varying amounts of heparin (right panel).

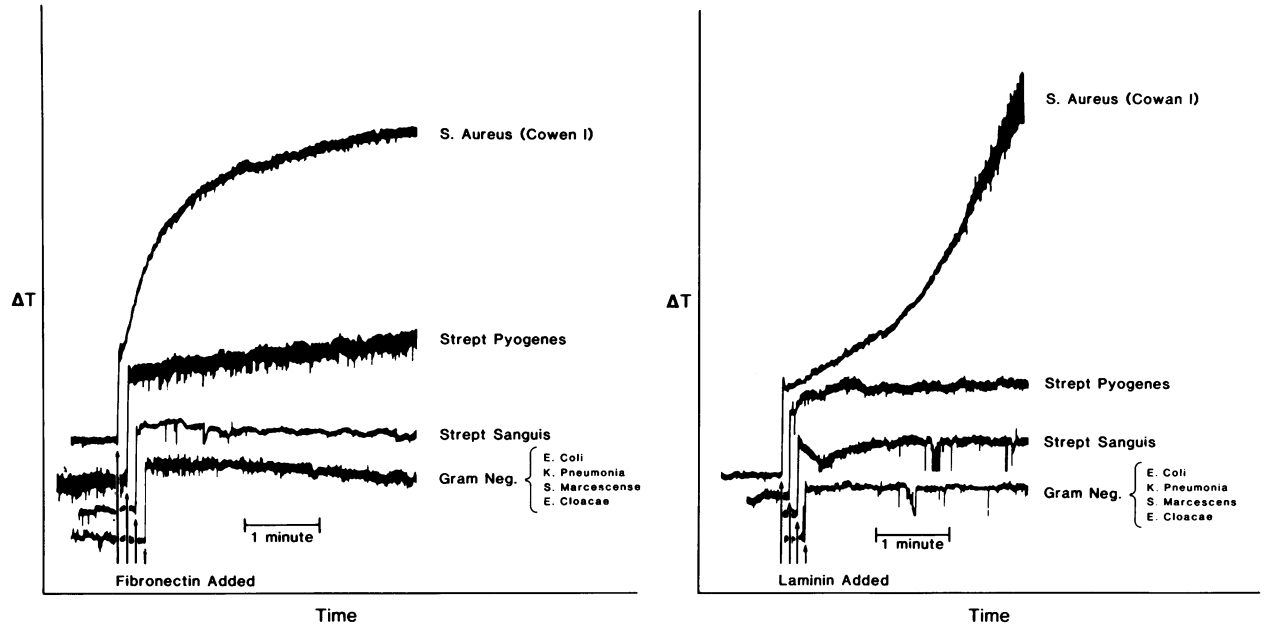

Figure 6—Bacterial aggregation to fibronectin and laminin. Fifty microliters of fibronectin (2 mg/ml) was added to 450 μl 2 × 10^9/ml *S aureus* Cowan I, *S pyogenes*, *S anguis*, and gram-negative bacteria including *E coli*, *K pneumoniae*, *S marcescens*, and *E cloacae* (left panel). Similarly (right panel), 50 μl laminin (1 mg/ml) was added to each bacterial suspension.

be seen that binding to laminin, fibronectin, or Type IV collagen was not dependent on the teichoic acid content of the *S aureus*, because a teichoic-acid-deficient strain bound well to these substrates. Interestingly, the protein A content of the *S aureus* was important in binding to laminin and Type IV collagen, but not to fibronectin. Both streptococcal strains tested, *S pyogenes* and *S sanguis*, bound moderately well to both laminin and fibronectin. Gram-negative organisms did not bind well to laminin, fibronectin, or Type IV collagen, showing not more than 1-2% of the added bacteria adhering to the coated substrates, which is tenfold less than that observed with staphylococci or streptococci. This correlates with the aggregation data, in that none of the gram-negative organisms aggregated to the matrix proteins when in suspension. Finally, trypsin pretreatment of *S aureus* greatly reduced the binding of these bacteria to fibronectin (7% ± 1%), laminin (4% ± 1%), and Type IV collagen (5% ± 2%), compared with control bacteria that were not treated with proteases.

Discussion

The two *in vitro* assays used in this study demonstrated a specific interaction between *S aureus* and the cell matrix proteins fibronectin, laminin, and Type IV collagen. *S aureus* (Cowan I) aggregated in a dose-dependent manner in the presence of the noncollagenous basement membrane matrix proteins laminin and fibronectin as well as Type IV collagen. The studies also show bacterial aggregation with fibronectin and use a novel technique for assessment of this, ie, the utilization of a platelet aggregometer. Previous studies have shown fibronectin to cause clumping of *S aureus*[8,9] but not *S epidermidis*. In addition, these studies demonstrate the ability of laminin and Type IV collagen to induce *S aureus* aggregation. Aggregation was apparently influenced by a protein(s) on the staphylococcal surface, because the trypsinized Cowan I strain did not aggregate in the presence of laminin, fibronectin, or Type IV collagen. However, a teichoic-acid-deficient strain did aggregate when fibronectin was added, but not Type IV collagen or laminin. Heparin decreased the rate but did not completely block the aggregation of *S aureus* in response to fibronectin, laminin, or Type IV collagen. It has been shown that fibronectin binds to *S aureus* via the 27 kd amino terminal region.[5] The 27 kd fragment of fibronectin has weak heparin binding activity,[27] and Vanderwater demonstrated a 60% inhibition of fibronectin binding to *S aureus* with high concentrations of heparin. Laminin[32] also has the ability to bind heparin. Our data suggest that heparin can decrease the rate of matrix-protein-induced aggregation of *S aureus* but does not completely block it. Heparin may be interacting stoichiometrically with bacterial binding sites on these molecules or may be altering bacterial surface charge by its anionic nature. Alternatively, the binding of heparin to one site may modify an alternate site on the fibronectin molecule that is more specifically responsible for bacterial adhesion. The abil-

Table 1—Bacterial Adherence to Laminin, Fibronectin, and Type IV Collagen

	Laminin	(n)	Fibronectin	(n)	Type IV collagen	(n)
S aureus (Cowan 1)	22% ± 2%	(20)	23% ± 2%	(20)	17.0% ± 3%	(16)
S aureus (trypsin-treated)	4% ± 1%	(8)	7% ± 1%	(8)	1.0% ± 1%	(8)
S aureus (52A5) (teichoic-acid-deficient)	18% ± 2%	(6)	25% ± 3%	(6)	22.0% ± 1%	(4)
S aureus (Wood 46) protein-A-deficient)	1% ± 0.5%	(6)	19% ± 2%	(6)	3.2% ± 1%	(8)
S pyogenes	18% ± 2%	(9)	19% ± 2%	(6)	ND	
S sanguis	14% ± 2%	(9)	11% ± 2%	(8)	ND	
E coli (ON2)	2%	(15)	1%	(10)	1%	(8)
E cloacae	2%	(6)	1%	(6)	1%	(8)
K pneumoniae	2%	(6)	1%	(6)	1%	(8)
S marcescens	2%	(6)	1%	(6)	1%	(8)

Data are the mean ± SE calculated as in Materials and Methods. ND, not determined

ity to aggregate seemed to be relatively unique to *S aureus*, because other gram-positive and gram-negative organisms did not aggregate in response to these proteins.

S aureus Cowan I also bound tightly to surfaces coated with laminin, Type IV collagen, and fibronectin in an adhesion assay. A blood culture of *S aureus* from a patient with endocarditis also bound to these substrates. There were differences in the binding of various *S aureus* strains to these substrates. The Wood 46 strain, which is relatively deficient in protein A, bound well to fibronectin but poorly to laminin or Type IV collagen. Previous studies have shown that the "receptor" for fibronectin on *S aureus* is a protein,[10,11] but it is apparently not protein A.[33,34] Laminin and Type IV collagen, however, may interact with staphylococcal surface protein A to enhance their adhesion, and this possibility is under investigation. Lipoteichoic acid is important for binding fibronectin to streptococci,[12] and teichoic acid has been purported to be the receptor for fibronectin on *S aureus*.[35] In contrast, in these studies, teichoic acid did not seem to be essential in the cell wall of the *S aureus* for binding to laminin, fibronectin, or Type IV collagen, because the teichoic-acid-deficient strain of *S aureus* bound extremely well. The gram-positive streptococci also bound well to laminin and fibronectin. Fibronectin binding to group A streptococci is consistent with the findings of previously published reports.[13,36] Gram-negative organisms adhere very poorly to surfaces coated with laminin, Type IV collagen, and fibronectin. Several investigators have shown that fibronectin does not bind avidly to gram-negative organisms.[24,34] However, Speziale has reported that laminin interacts with fimbriae or pili of *E coli*.[26] In their study they used radioactive laminin to study the binding of laminin to fimbriated *E coli*. These studies did not use fimbriated *E coli* or other piliated gram-negative organisms, which may explain the differences in our results. Our data are in conflict with Speziale's with regard to *S aureus*: he did not find laminin binding to this organism. Methodologic considerations may account for these differences. For example, our studies evaluated the active binding of bacteria to laminin, rather than the converse. It is possible that a small population of normal or damaged bacteria might bind to laminin, which could account for the data regarding radiolabeled laminin binding to gram-negative organisms. Finally, certain organisms adhered well to matrix-protein-coated wells but did not aggregate in response to these proteins. This finding may reflect physical differences in the interaction of bacteria with these proteins when in solution or unsolubilized on a surface. Fibronectin may change its conformation in a variety of physiologic situations.[37] We speculate that the differences in soluble and insoluble tissue matrix protein may affect the interaction with bacteria and may account for the differing roles these proteins could play in infection pathogenesis.

These studies describe interactions of cell matrix proteins with a variety of bacteria with a novel technique involving nephelometry and with assessment of bacterial adherence. We speculate that the exposure of these matrix proteins during tissue injury may allow bacteria to bind, aggregate, and assemble for tissue invasion. It is possible that aggregation may allow the organisms to elude phagocytic host defenses. Proctor[38] has demonstrated that the invasiveness of *S aureus* is correlated with the bacteria's ability to agglutinate in the presence of fibronectin. Further studies are needed to clarify the role of matrix proteins in host defense and injury during bacterial infections.

References

1. Peterson PK, Quie PG: Bacterial surface components and

the pathogenesis of infectious diseases. Ann Rev Med 1981, 32:29-43
2. Costerton JW, Geesey GG, Cheng KJ: How bacteria stick. Sci Am 1978, 238:86-95
3. Beachey EH, Ofek I: Epithelial cell binding of group A streptococci by lipoteichoic acid on fimbriae denuded of M protein. J Exp Med 1976, 143:759-771
4. Ofek I, Beachey EH: Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect Immun 1978, 22: 247-254
5. Mosher DF, Proctor RA: Binding and factor XIIIa-mediated cross-linking of a 27-kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science 1980, 209:927-929
6. Proctor DE, Mosher DF, Olbrantz J: Fibronectin binding of *Staphylococcus aureus*. J Biol Chem 1982, 257: 14788-14794
7. Van De Water L, Destree AT, Hynes RO: Fibronectin binds to some bacteria but does not promote their uptake by phagocytic cells. Science 1983, 220:201-204
8. Espersen F, Clemmensen I: Clumping of *Staphylococcus aureus* by human fibronectin. Acta Pathol Microbiol Immunol Scand [B] 1981, 89:317-321
9. Mosher DF: Fibronectin. Prog Hemost Thromb 1980, 5:111-151
10. Switalski LM, Ryden C, Rubin K, Ljungh A, Hook M, Wadstrom T: Binding of fibronectin to *Staphylococcus* strains. Infect Immun 1983, 42:628-633
11. Espersen F, Clemmensen I: Isolation of a fibronectin binding protein from *Staphylococcus aureus*. Infect Immun 1982, 37:526-531
12. Courtney HS, Simpson WA, Beachey EH: Binding of Streptococcal lipoteichoic acid to fatty acid-binding sites on human plasma fibronectin. J Bacteriol 1983, 153: 763-770
13. Simpson WA, Hasty DL, Mason JM, Beachy EH: Fibronectin-mediated attachment of group A streptococci to human PMNs. Infect Immun 1982, 37:805-810
14. Abraham SN, Beachey EH, Simpson WA: Adherence of *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* to fibronectin-coated and uncoated epithelial cells. Infect Immun 1983, 41:1261-1268
15. Woods DE, Straus DC, Johanson WG, Bass JA: Role of fibronectin in the prevention of adherence of *Pseudomonas aeruginosa* to buccal cells. J Infect Dis 1981, 143: 784-790
16. Vercellotti GM, Lussenhop D, Peterson PK, Furcht LT, McCarthy JP, Jacob HS, Moldow CF: Bacterial adherence to fibronectin and endothelial cells: A possible mechanism for bacterial tissue tropism. J Lab Clin Med 1984, 103:34-43
17. Yamada KM: Cell surface interactions with extracellular materials. Annu Rev Biochem 1983, 52:761-799
18. Timpl R, Martin GR: Components of basement membranes, Immunochemistry of the Extracellular Matrix. Edited by H Furthmayer. Boca Raton, Fla, CRC Press, 1982, pp 119-150
19. Terranova VP, Rohrbach DH, Martin GR: Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. Cell 1980, 22:719-726
20. Vlodavsky I, Gospodarowicz D: Respective roles of laminin and fibronectin in adhesion of human carcinoma and sarcoma cells. Nature 1081, 289:304-306
21. Couchman JR, Hook M, Rees DA, Timpl R: Adhesion, growth and matrix production by fibroblasts on laminin subunits. J Cell Biol 1983, 96:177-183
22. McCarthy JB, Palm SL, Furcht LT: Migration by heptotaxis of a Schwann cell tumor line to the basement membrane glycoprotein laminin. J Cell Biol 1983, 97:772-777
23. McCarthy JB, Furcht LT: Laminin and fibronectin promote the haptotactic migration of B16 mouse melanoma cells in vitro. J Cell Biol 1984, 98:1474-1480
24. Martinez-Hernandez A, Amenta PS: The basement membrane in pathology. Lab Invest 1983, 48:656-677
25. Timpl R, Weidemann H, Van Delden V, Furthmayer H, Kuhn K: A network model for the organization of Type IV collagen molecules in basement membranes. Eur J Biochem 1981, 120:203-211
26. Speziale P, Hook M, Wadstrom T, Timpl R: Binding of the basement membrane protein laminin to *E. coli*. FEBS Lett 1982, 146:55-58
27. Smith DE, Furcht LT: Localization of two unique heparin binding domains of human plasma fibronectin with monoclonal antibodies. J Biol Chem 1982, 257:6518-6523
28. Palm SL, Furcht LT: Production of laminin and fibronectin by Schwannoma cells: Cell-protein interactions *in vitro* and protein localization in peripheral nerve *in vivo*. J Cell Biol 1983, 96:1218-1226
29. Kleinman HK, McGarvey ML, Hassell JR, Martin GR: Formation of a supramolecular complex is involved in the reconstitution of basement membrane components. Biochemistry 1983, 22:4969-4974
30. Kleinman HK, McGarvey ML, Liotta LA, Robey PG, Tryggvason K, Martin GR: Isolation and characterization of Type IV procollagen, laminin and heparan sulfate proteoglycan from the EHS sarcoma. Biochemistry 1982, 24:6188-6193
31. Laemmli UK: Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 1970, 227: 680-685
32. Sakashita S, Engvall E, Ruoslahti E: Basement membrane glycoprotein laminin binds to heparin. FEBS Lett 1980, 116:243-246
33. Ryden C, Rubin K, Speziale P, Hook M, Lindberg M, Wadstrom T: Fibronectin receptors from *Staphylococcus aureus*. J Biol Chem 1983, 258:3396-3401
34. Verbrugh HA, Peterson PK, Smith DE, Nguyen BT, Hoidal JR, Wilkinson BJ, Verhoef J, Furcht LT: Human fibronectin binding to staphylococcal surface protein and its relative inefficiency in promoting phagocytosis by human PMNs, monocytes, and alveolar macrophages. Infect Immun 1981, 33:811-819
35. Bibel DJ, Aly R, Shinefield HR, Maibach HI: The *Staphylococcus aureus* receptor for fibronectin. J Invest Dermatol 1983, 80:494-496
36. Simpson WA, Beachey EH: Adherence group A streptococci to fibronectin on oral epithelial cells. Infect Immun 1983, 39:275-279
37. Williams EC, Janmey PA, Ferry JD, Mosher DF: Conformational states of fibronectin: Effects of pH, ionic strength and collagen binding. J Biol Chem 1982, 257: 14973-14978
38. Proctor RA, Christman G, Mosher DF: Fibronectin-induced agglutination of Staphylococcus aureus correlates with invasiveness. J Lab Clin Med 1984, 104:455-469

Acknowledgments

We thank Debra Armstrong for technical assistance and Carol Taubert and Suzanne Marshall for assistance with manuscript preparation.